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(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 85305660.4

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AT BE CH DE FR GB IT LI LU NL SE

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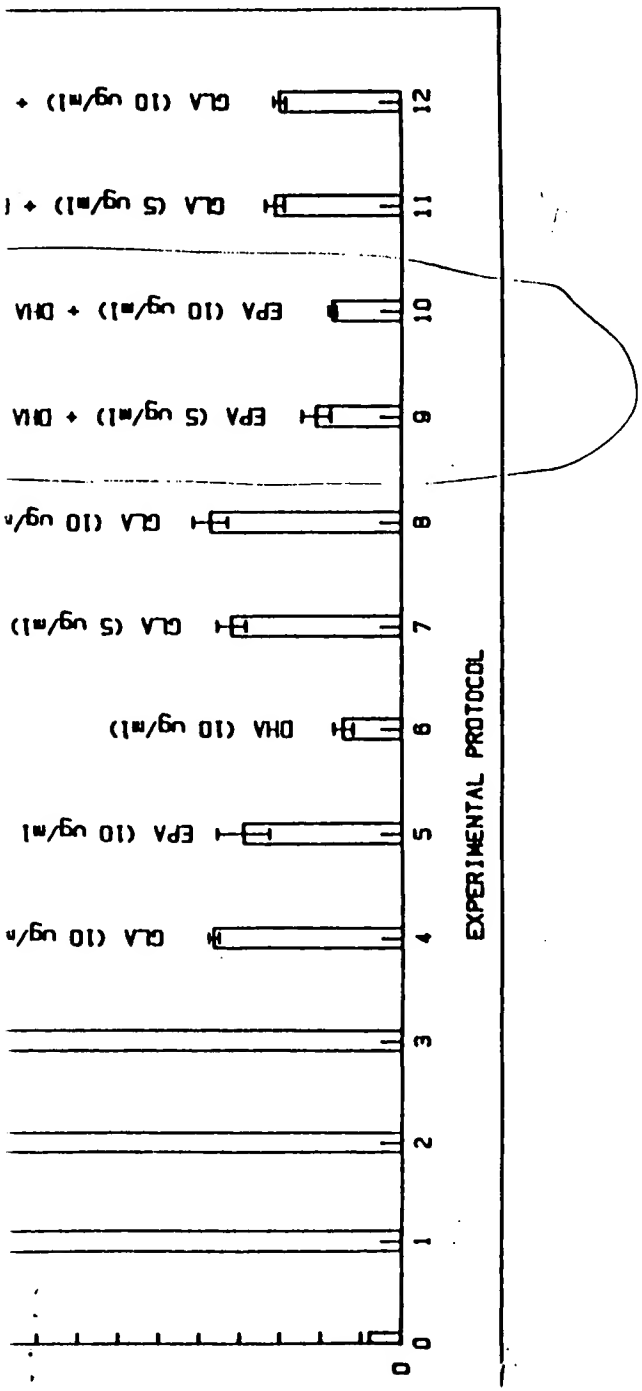
(54) ···osanoids for use in cancer therapy.

(57) The invention comprises a method of normalising cellular eicosanoid balance by administering to a warm blooded animal an effective amount of a composition chosen from the group comprising eicosapentanoic acid (EPA), docosahexanoic acid (DHA) a mixture of EPA and DHA and a mixture of EPA, DHA and GLA. The invention also relates to compositions for normalising cellular eicosanoid balance for the prevention or treatment of cancer.



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The features disclosed in the foregoing description, in the following claims may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

\N SEARCH REPORT

ELEVANT		CLASSIFICATION OF THE APPLICATION (Int Cl 4)
c. of relevant	Relevant to claim	
	4-9	
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encla- r eico- e latter hexanoic , and		TECHNICAL FIELDS SEARCHED (Int Cl 4)

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

0175468
Application number

EP 85 30 5660

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
X	PROSTAGLANDINS LEUKOTRIENES AND MEDICINE, vol. 15, no. 1, July 1984 pages 15-33 J. BOOYENS et al.: "Some effects of the essential fatty acids linoleic acid and alpha-linoleic acid and of their metabolites gamma-linoleic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and of prostaglandins A_1 and E_1 on the proliferation of human osteogenic sarcoma cells in culture." * Whole article * ---	4-9	A 61 K 31/20
X	BE-A- 897 806 (SENTRACHEM) * Whole document * ---	4-9	
X	DE-A-3 334 323 (SENTRACHEM) * Whole document * ---	4-9	TECHNICAL FIELDS SEARCHED (Int Cl 4) A 61 K 31/00

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INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely:

Claims searched incompletely: 4-9 : Reason for limitation of the search: see page 2.

Claims not searched: 1-3

Reason for the limitation of the search

For claims 1-3:

Method for treatment of the human or animal body by surgery or therapy (see art. 52(4) of the European Patent Convention).

Place of search	Date of completion of the search	Examiner
The Hague	08-04-1987	THEUNS

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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nistrating to a warm blooded
f a composition including an
tance chosen from the group
acid (EPA), docosahexanoic
A and DHA, and a mixture of

laim 1 in which the composition
daily administration of less than
nt or ingredients per 50 to

hich the substances are in the
zinc salts.

ing cellular eicosanoid balance
tment of cancer including a

a composition having an effective amount of a substance chosen from the group comprising eicosapentanoic acid (EPA), docosahexanoic acid (DHA) a mixture of EPA and DHA, and a mixture of EPA, DHA and GLA.

5. DHA, or a pharmaceutically acceptable salt thereof or DHA or a pharmaceutically acceptable salt thereof with EPA or a pharmaceutically acceptable salt thereof and/or GLA or a pharmaceutically acceptable salt thereof for use as an active therapeutic substance.

6. DHA, or a pharmaceutically acceptable salt thereof or DHA or a pharmaceutically acceptable salt thereof with EPA or a pharmaceutically acceptable salt thereof and/or GLA or a pharmaceutically acceptable salt thereof for use in the prevention or treatment of cancer.

7. The use of DHA or DHA with EPA and/or GLA in the manufacture of a medicament to prevent or treat cancer.

8. The use of DHA or DHA with EPA and/or GLA in the manufacture of a medicament to prevent or treat cellular eicosanoid imbalance.

9. A use according to claim 7 or 8, wherein a pharmaceutically acceptable salt of one or more of DHA, EPA and GLA is used.

FIELD OF THE INVENTION

This invention relates to substances and compositions containing such substances for use in the treatment of cancerous conditions.

BACKGROUND

In 1980 Horrobin (The Reversibility of Cancer: The Relevance of Cyclic AMP, Calcium, Essential Fatty Acids and Prostaglandin E_1 Med. Hypotheses 1980, Vol. 6, pages 469 to 486) dealt extensively with metabolic abnormalities common to almost all cancer cells, and with possible causative factors for these. Horrobin concludes that a metabolic abnormality in the synthesis of the prostaglandins thromboxande A_2 (TXA_2) and prostaglandin E_1 (PGI_1) is the final factor which allows an initiated cancer cell to express its abnormality, that is to divide ad infinitum. Horrobin further proposed (on the basis of evidence present in the general literature) that the defect which leads to the abnormality in the synthesis of TXA_2 and PGE_1 is an inhibition of the enzyme delta-6-desaturase. This enzyme converts the essential fatty acid linolenic acid (LA), to gamma linolenic acid (GLA) in all normal cells of the body. GLA is further metabolised to dihomo-gamma-linolenic acid (DGLA) which in turn is converted to prostaglandins of the 1-series, which includes PGI_1.

at PGE_1 and TXA_2 are potent
rs of the biochemistry of all
TXA_2 however cannot function
. Horrobin surmised that a
thus disabled TXA_2 will cause
the cell, of sufficient magnitude
olled division of potential

that inter alia a GLA supplement
cer patients receiving conventional
test his hypothesis, namely, that
olic block caused by an inhibited
6-d) activity, it should be
cancer cells by reverse trans-

lication 0 037 Horrobin claims a
oproline for the treament of

present invention to provide
eatment of cancer by taking into
ction of one or more normalised

According to the invention a method of normalising cellular eicosanoid balance by administration of eicosapentanoic acid (EPA) and/or docosahexanoic acid (DHA).

In a preferred form of the invention EPA or DHA or a mixture of EPA and DHA is administered in a number of possible forms such as, for example, capsules , tablets or other convention pharmaceutical forms, or in admixture with foodstuffs, beverages and the like.

It will be appreciated that suitable salts, derivatives, or chemical analogues of the above substances are also in the scope of the present invention. In particular the magnesium and zinc salts are important.

The substance or composition may be provided in unit dosage form, eg for daily or twice-daily administration, such as in tablets or capsules. In each capsule the active ingredient may be solution, as described above, or it may be in the form of a tablet or particulate mixture, comprising the active ingredient together with a solid diluent or carrier. A unit dosage for daily

ally for a person of [illegible] to 100
contain up to 1000 mg of active

r the active ingredients comprise
saline solutions or any other
vents suitable for human intake.

ow be described and illustrated
ing examples which includes
vivo studies:

CANCER CELLS IN CULTURE

coma cells were seeded into 50
lasks and maintained in the

red for three weeks in the presence

only - control
& 10 1 Na_2CO_3/ml added every
& 20 g EPA/ml medium added every
& 20 g oleic acid/ml added every
&. 5 g PGE_1/ml added every second

011540

vi) growth medium & 5 g PGA_1/ml added every second day

vii) growth medium & 5 g PGF_{1a}/ml added every second day.

At the end of three weeks the culture flasks were stained with a 0,1% Amidoschwarz stain solution.

RESULTS

At the end of the three weeks period the initially seeded osteogenic sarcoma cells had established colonies of various sizes almost covering the entire floor of the culture flasks of the control and the Na_2CO_3 supplemented flasks.

Oleic acid supplemented cultures achieved much greater growth as control cultures.

PGE_1 and PGA_1 cultures achieved about 25% of the growth of control cultures.

PGI_{1a} cultures achieved about the same growth as the control cultures.

The EPA supplemented cultures were completely devoid of any colonies in 500, 1 000 and 2 000 cell density cultures.

more pronounced growth suppressive
had no effect at all on cancer cell
. EPA had a complete growth

ggest that uncontrolled cell division
d be the result of abnormalities in
some of the prostaglandins in such
m a block in their synthesis from
s. Such abnormalities are evidently
ing the cancer cells with EPA, the
the required prostaglandins can
eir required concentrations. Once
by cancer cells, their uncontrolled
ly totally checked.

ising observation reported in
(the essential fatty acid inter-
precursor of the 3-series prosta-
in, thromboxane A3 and leukotrienes,
- EPA being derived from the
sential fatty acid γ-linolenic
he action of d-6-d to give
id (C 18:4 W3), which undergoes
icosatetraenoic acid (C20:4 W3),
ise to eicosapentaenoic acid
of delta-5-desaturase) supplement-

ation by 40 g/ml medium of mg63 osteogenic sarcoma cells completely suppresses proliferation and colony formation of the cells in culture, this experiment was repeated in order to confirm the observation.

In addition the final product of γ-linolenic acid metabolism, which is DCAA was also added to osteogenic cells in culture.

PROCEDURE

MG63 Human osteogenic sarcoma cells were seeded in culture flasks as described in example 1. 2 000 cells were seeded in each flask. Duplicate sets of flasks were used for each of the fatty acids tested. The following fatty acids dissolved in standard growth medium were added to the cells in culture, after allowing 2 days for cell attachment, and again after a further 3 days. Each culture therefore had only 2 additions of the relevant fatty acid. The cells were stained and examined at the end of 7 days in culture.

1. Culture medium only control.
2. 5, 10, 20, 40, 60, 80 and 100 g oleic acid respectively /ml culture medium (Oleic acid (OA) is an 18 C fatty acid with one unsaturated bond in the omega-9 position. It is there are structurally nearly identical to either LA and γ LA with the

the number of double bonds
cule. On account of the latter
e LA and γ-LA cannot give rise
s therefore considered to be
id to use as a control when
ects of the eicosanoids.

and 100 g/ml EPA respectively

and 100 g/ml DHA respecitvely

achieved greaterdensities of
ls of supplementation between
as did the controls with

d an almost equal, progressive,
the proliferation and colony
osteogenic carcinoma cells.

ly suppressed cell growth and
levels of supplementation above
um.

colony of cells could be found
nation of the cultures which

had been supplemented with either EPA or DHA at supplementation levels between 10 and 100 g/ml.

It would therefore appear that the fatty acid metabolites, EPA and DHA have the ability to individually arrest and suppress cancer cell growth. It would further appear that any one of these eicosanoid precursor fatty acids separately or in combination could be used for the treatment of cancer.

These results have been confirmed using three other cancer cell types i.e. larynx carcinoma
hepatoma (liver cancer)
melanoma (skin cancer).

TABLE 1

The effect of supplementing human larynx carcinoma cells in culture with varying concentrations of oleic acid and eicosapentaenoic acid on the rate of proliferation. Cells were seeded in a concentration of $0,0698 \times 10^6$/ml on day 1 of the experiment. Growth media containing the various fatty acid supplements were added to the cultures on days 3 and 5 of the experimental period and cell counts were made on day 8 of the experimental period. Control cultures received standard growth medium only.

SD	p-value	Difference between control and supplemented counts
0,057		
0,163	0,05	ns
0,091	0,05	ns
0,49	0,05	ns
0,42	0,05	ns
0,015	0,01	hs
0,0357	0,01	hs

11

human larynx carcinoma cells in
n equal proportion of
osapentanoic acid;
rostaglandins F_1 and F_2 ; and
n varying concentrations on the
ls were seeded in a concentration
of the experiment. The various
lays 3 and 5 of the experimental
e made on day 8.

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Suppiement and concentration g/ml medium	Mean Cell count x 10^6	SD	p-value	Difference between control and supplemented counts
Control	0,33	0,006		
GLA) 20 +)	0,22	0,005	0,01	hs
EPA) 60	0,09	0,009	0,01	hs
PGE_1) +) 5 PGA_1)	0,19	0,016	0,01	hs
PGE_1) +) 5 PGF_2)	0,33	0,009	0,05	ns
EPA) 20 + DHA)40	0,13	0,018	0,01	hs
DHA)60	0,06	0,005	0,01	hs
	0,009	0,003	0,01	hs

Results in respect of hepatoma and melanoma were very similar to the above experiments on larynx carcinoma

In all of the above experiments, duplicate experiments were conducted using normal MDBK cells in culture. It is important to note that none of the EFA's

tients who were described
failure of conventional
ion therapeutic procedures
to daily dietary supple-
g EPA + 0,5g DHA daily).
al patients are being continued.

about 55) was suffering from
ed a terminal case). He was
upplement as described above,
his oesophagus and massive
cavity. After six months,
back at work.

ering from a brain
was recommended and he was
than a month.

s diet was supplemented with
d systematically and is now
own car. The tumour diameter
still regressing.

arge primary liver cancer.
supplemented with EPA/ DHA /GLA.
still regressing substantially
rimary liver cancer patients
f about 40 days post positive

Subject D (age 60) suffered from unilateral larynx carcinoma and was expected to live for not more than a few months. He is still (after more than a year) receiving a dietary supplement of EPA/ DHA /GLA and there has been total regression of the nodule and D is leading a normal life.

In two examples, subjects E and F (ages 55 and 50) suffering from mesothelioma were both given only a short while to live. They are now apparently healthy following six months of dietary supplement.

Further experiments were conducted in relation to the effect of EPA, DHA and mixtures thereof, and such mixtures with GLA and were compared with controls and also with GLA on its own. The results of these experiments are given in the following Table.